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20/Brief

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of	:	
GEORGE J. MURAKAWA, ET AL.	:	Group Art Unit: 1814
Filed: September 1, 1989	:	Examiner: M. Escallon
Serial No.: 07/402,450	:	
FOR: METHOD FOR AMPLIFICATION AND DETECTION OF RNA SEQUENCES	:	

113

93-4018

APPELLANTS' BRIEF

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

STATUS OF CLAIMS

Claims 1-17 and 26-30 have been cancelled.

Claims 18-25 and 31-33 are pending.

Claims 18-25 and 31-33 are appealed.

The appealed claims 18-25 and 31-33 are reproduced in the Appendix.

Claim 18, as amended by a concurrently filed amendment pursuant to 37 C.F.R. §1.116(b), is also reproduced in the Appendix.

STATUS OF AMENDMENTS

An Amendment Pursuant to 37 C.F.R. §1.116(b) is concurrently filed. This amendment is intended to clarify claim 18 and to adopt one suggestion by the Examiner (Final Action, page 3) thus

avoiding the objection and rejection under 35 U.S.C. §112, first paragraph and rejection of claim 18 under 35 U.S.C. §112, second paragraph set forth in the final action at pages 2-4.

SUMMARY OF INVENTION

The invention is a process for detecting the presence of false positive data or false negative data in a hybridization assay for a target viral sequence. A sample and a synthetic RNA reference sequence are concurrently PCR amplified. Probes specific to the target sequence and the reference sequence are applied to the coamplification product.

The manner in which false positive or false negative data is detected by the claimed process is apparent from the following table:

Result	Target	Reference Sequence	
1	+	+	Positive
2	0	+	Negative
3	0	0	False Negative[1/]
4	+	0	False Positive

1/ The reference probe would have hybridized if the assay were functioning correctly. It did not, hence the negative result for the target is false.

ISSUES

1. Whether the appealed claims define inventions which are made obvious, 35 U.S.C. §103, by Mullis, et al. 4,683,195 in view of Ratner, et al. Nature 313(2):277 (1985).

The final rejection states:

> Claims 18-23 and 31-33 are again rejected under 35 U.S.C. §103 as being unpatentable over mullis et al. in view of Ratner et al.

This rejection is essentially identical to that set forth in the last Office Action and will not be repeated. Applicants' arguments have been fully considered but they are not deemed to be persuasive. Applicants argues [sic] that Mullis et al. do not disclose "reference sequence" contrary to applicants' assertion Mullis et al. disclose the amplification of viral RNA in the bridging sentences between columns 7 and 8. It is disclosed the alternative adding of either one or two primers so as to facilitate the amplification second strand target sequence, followed by amplification of both strands by two primers, in column 9, lines 5-49. The whole process is summarized by Mullis et al. in column 2, line 63 through column 3, line 33, wherein the detection step with a labeled probe is cited in column 3, lines 25-27. Mullis et al. is a general but very detailed teaching as to the PCR method. Ratner et al. give the complete HIV genomic sequence as well as discussion of regions contained therein which clearly therefore gives the required knowledge for not only many possible primer sequences but probes as well.

Therefore, it would have been obvious to one of ordinary skill in the art at the time of the instant invention to apply the PCR technique of Mullis et al. to HIV amplification and detection because Mullis et al. supply the general technique with a great deal of guidance as to its application and Ratner et al. supplies the sequence information which is the last required data for the use of PCR in HIV amplification and detection. [pp. 4-5]

2. Whether claims 24 and 25 define inventions that are made obvious by Mullis in view of Ratner and further in view of Hennighausen et al. EMBO J. 5(6):1367 (1986) and Wathen, et al. J. Virol. 41(2):462 (1982).

The final rejection states:

Hennighausen et al. give the complete HCMV immediate early (IE1) genomic sequence. Wathen et al. give probes that hybridize late HCMV genes. Therefore as applied above, it would have been obvious to someone of ordinary skill in the art at the time of the instant invention to apply the PCR technique of Mullis et al. to HMCV amplification and detection because Mullis et al. supply the technique with a great deal of guidance and Hennighausen et al. and Waten [sic] et al. supply the sequence information which is the last required data for use of PCR in HCMV (IE) or late regions for amplification and detection. [p. 6]

3. Whether the specification is properly subject to objection and claims 18-25 and 31-33 are properly subject to rejection under 35 U.S.C. §112, first paragraph.

4. Whether claim 18 is properly rejected under 35 U.S.C. §112, second paragraph.

5. Whether claims 18-30 are subject to a provisional obviousness-type double patenting rejection over claims 24-53 of copending application Serial No. 07/180,740.

This issue, as stated, is moot. Claims 24-53 have been cancelled in Serial No. 07/180,740.

GROUPING OF CLAIMS

The appealed claims 18-25 and 31-33 stand or fall together.

ARGUMENT

Issues 1 and 2--Rejection of claims 18-23 and 31-33 under §103 over Mullis in view of Ratner; rejection of claims 24 and 25 under §103 over Mullis in view of Ratner, Hennighausen and Wathen.

(i) The error in this rejection is that the portions of Mullis relied upon in the final action do not provide any "RNA reference sequence" as required by step (ii)(b) of claim 18 either as finally rejected or as amended pursuant to 37 C.F.R. §1.116(b). Nor is there any suggestion in Mullis of steps (iv) and (v) of claim 18 (as finally rejected or as amended pursuant to 37 C.F.R. §1.116(b)).

The claimed invention is not merely "apply[ing] the PCR technique of Mullis to HIV amplification and detection" (Final Action, p. 5). Actually, no method for detecting false positive or negative data is taught or made obvious by Mullis.

The portions of Mullis relied upon by the final action in correct full text state:

> More specifically, the present invention provides a process for detecting the presence or absence of at least one specific nucleic acid sequence in a sample containing a nucleic acid or mixture of nucleic acids, or distinguishing between two different forms of sequences in said sample, wherein the sample is suspected of containing said sequence or sequences, which process comprises:
>
> (a) treating the sample with one oligonucleotide primer for each strand of each different specific sequence suspected of being present in the sample, under hybridizing conditions such that for each strand of each different sequence to be detected an extension product of each primer is synthesized which is complementary to each nucleic acid strand, wherein said primer or primers are selected so as to be substantially complementary to each strand of each specific sequence such that the extension product synthesized from one primer, when it is separated from its complement, can serve as a template for synthesis of the extension product of the other primer;
>
> (b) treating the sample under denaturing conditions to separate the primer extension products from their templates if the sequence or sequences to be detected are present;
>
> (c) treating the sample with oligonucleotides primers such that a primer extension product is synthesized using each of the single strands produced in step (b) as a template, resulting in amplification of the specific nucleic acid sequence or sequences if present;
>
> (d) adding to the product of step (c) a labeled probe capable of hybridizing to said sequence being detected or a mutation thereof; and
>
> (e) determining whether said hybridization has occurred.

The steps (a)-(c) may be conducted sequentially or simultaneously. In addition, steps (b) and (c) may be repeated until the desired level of sequence amplification is obtained. [Col. 2, l. 63-Col. 3, l. 33]

The sentence bridging columns 7 and 8 states:

The nucleic acid or acids may be obtained from any source, for example, from plasmids such as pBR322, from cloned DNA or RNA, or from natural DNA or RNA from any source, including bacteria, yeast, viruses, and higher organism is such as plants or animals. DNA or RNA may be extracted from blood, tissue material such as chorionic villi or amniotic cells by a variety of techniques such as that described by Maniatis et al., Molecular Cloning: A Laboratory Manual, (New York: Cold Spring Harbor Laboratory, 1982), pp. 280-281. [Col. 7, l. 66-Col. 8, l. 8]

Column 9, lines 5-49 state:

If the original nucleic acid containing the sequence to be amplified is single stranded, its complement is synthesized by adding one or two oligonucleotide primers thereto. If an appropriate single primer is added, a primer extension product is synthesized in the presence of the primer, an agent for polymerization and the four nucleotides described below. The product will be partially complementary to the single-stranded nucleic acid and will hybridize with the nucleic acid strand to form a duplex of unequal length strands that may then be separated into single strands as described above to produce two single separated complementary strands. Alternatively, two appropriate primers may be added to the single-stranded nucleic acid and the reaction carried out.

If the original nucleic acid constitutes the sequence to be amplified, the primer extension product(s) produced will be completely complementary to the strands of the original nucleic acid and will hybridize therewith to form a duplex of equal length strands to be separated into single-stranded molecules.

When the complementary strands of the nucleic acid or acids are separated, whether the nucleic acid was originally double or single stranded, the strands are ready to be used as a template for the synthesis of additional nucleic acid strands. This synthesis can be performed using any suitable method. Generally it occurs in a buffered aqueous solution, preferably at a pH of 7-9, most preferably about 8. Preferably, a molar excess (for cloned nucleic acid, usually about 1000:1 primer:-template, and for genomic

> nucleic acid, usually about 10^6:1 primer:template) of the two oligonucleotide primers added to the buffer containing the separated template strands. It is understood, however that the amount of complementary strand may not be known if the process herein is used for diagnostic applications, so that the amount of primer relative to the amount of complementary strand cannot be determined with certainty. As a practical matter, however, the amount of primer added will generally be in molar excess over the amount of complementary strand (template) when the sequence to be amplified is contained in a mixture of complicated long-chain nucleic acid strands. A large molar excess if preferred to improve the efficiency of the process.

These quoted portions of Mullis merely describe concurrent PCR amplifications and the use of "a labelled probe" to detect an amplified preselected sequence or a mutation thereof.

More specifically, the generalization quoted from the final action mischaracterizes the claimed invention in a way that is essential to make out an "obviousness" argument.

Contrary to the generalization in the final action, the claimed invention requires simultaneous PCR amplification of viral RNA sample and at least one synthetic RNA sequence which does not include a preselected target sequence. The amplification product is denatured, e.g., in step (iii) of claim 18. The claim then requires a series of steps (iv) to (v)--or (vi) (see the 37 C.F.R. §1.116(b) amendment) all clearly absent from the reference teaching. Step (iv) and (v) of claim 18 as finally rejected state:

> (iv) subjecting said denatured amplification product or products of step (iii) to hybridization conditions separately and sequentially with probes homologous to said target sequence and to said reference sequence,

each of said probes being removed from a sequence with which it is hybridized prior to the separate and sequential subjection of said amplification products to hybridization with another of said probes;

(v) determining whether said amplified target and reference sequences are hybridized with said probes homologous therewith, false negative data being indicated by failure of said probes to hybridize either to the sample or to the reference sequence and false positive data being indicated by hybridization of the target sequence probe and by the absence of hybridization of the reference sequence probe.

The lack of clarity in these steps noted in the §112, second paragraph rejection has been addressed and, it is believed, avoided by the concurrently filed amendment under 37 C.F.R. §1.116(b).1/

The §103 rejection of claims 18-23 and 31-33 over Mullis in view of Ratner is inappropriate because of the failure of the primary reference to make obvious the process steps recited by claim 18. Hence the fact that Ratner may provide HIV sequence information is not material.

The rejection of claims 24 and 25 under §103 over Mullis in view of Ratner and further in view of Hennighausen and Wathen is inappropriate for the same reasons.

Issues 3 and 4--These issues arise under 35 U.S.C. §112, first and second paragraphs.

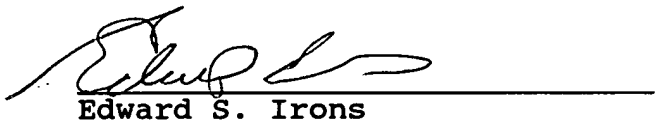
Counsel attempted to moot these issues by the concurrently filed amendment under 35 U.S.C. §1.116(b).

1/ These same steps (iv) and (v) are restated for clarification as steps (iv) to (vi) of claim 18 as it appears in the 37 C.F.R. §1.116(b) amendment.

CONCLUSION

The amendment under 37 C.F.R. §1.116(b) should be entered. This case should be passed to issue.

Respectfully submitted,



Edward S. Irons
Registration No. 16,541
919 18th Street, N.W.
Suite 800
Washington, D.C. 20006
(202) 785-6938

Dated: December 1, 1992

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APPENDIX

APPEALED CLAIMS 18-25 AND 31-33

18. A process for discerning false negative data or false positive data in the identification of a target viral RNA sequence in a peripheral blood cell sample which comprises:

(i) selecting said target viral RNA sequence;

(ii) simultaneously subjecting

(a) said sample and

(b) at least one synthetic RNA reference sequence which does not include said target sequence or which includes substantially more nucleotides than said target sequence or which includes at least about 20 nucleotides less than said target sequence

to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify said target sequence if present in said sample and said reference sequence;

(iii) denaturing the amplification product or products produced by step (ii);

(iv) subjecting said denatured amplification product or products of step (iii) to hybridization conditions separately and sequentially with probes homologous to said target sequence and to said reference sequence,

each of said probes being removed from a sequence with which it is hybridized prior to the separate and sequential subjection of said amplification products to hybridization with another of said probes;

(v) determining whether said amplified target and reference sequences are hybridized with said probes homologous therewith, false negative data being indicated by failure of said probes to hybridize either to the sample or to the reference sequence and false positive data being indicated by hybridization of the target sequence probe and by the absence of hybridization of the reference sequence probe.

19. A process as defined by claim 18 in which the reference nucleotide sequence utilized in step (ii) is

(i) a sequence present in the T-cell receptor expression product of cells infected by the virus containing said viral RNA;

(ii) a preselected RNA sequence present in substantially all of the cells of said sample,

(iii) a sequence including but having substantially more nucleotides than said target sequence by a multi-base insertion into a site in said viral RNA preselected with respect to said target sequence;

(iv) a beta actin sequence.

20. A process as defined by claim 18 in which said target viral sequence is located within the 3′ ORF region of HIV-1 and in which said reference sequence utilized in step (ii) is located in the constant region of the beta chain of the T-cell receptor expressed by T-4 lymphocytes infected by HIV-1.

21. A process as defined by claim 20 in which the reference sequence utilized in step (ii) is a beta actin sequence.

22. A process as defined by claim 20 in which the reference sequence utilized in step (ii) is a sequence formed by inserting a multi-base pair sequence into the 3' ORF region of HIV-1.

23. A process as defined by claim 18 or 19 in which at least one of the primers utilized in conducting the polymerase chain reaction in step (ii) includes the T-7 RNA polymerase sequence.

24. A process as defined by claim 18 in which said target viral sequence is in the HCMV major immediate early (IE) gene.

25. A process as defined by claim 18 in which said target viral sequence comprises RNA from the transcription of late HCMV genes.

31. A process as defined by claim 18 wherein a predetermined quantity of said reference sequence is used in step (ii)(b) and the probes utilized in step (iv) are labelled.

32. A process as defined by claim 31 in which the amount of signal obtained from the hybridized target sequence is compared with the amount of signal obtained from the hybridized predetermined quantity of said reference sequence.

33. A process as defined by claim 31 in which said probes utilized in step (iv) are labelled with an isotope or a flurorophore.

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